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ORIGINAL ARTICLE

Significant association between IL-18 and OCT4 gene polymorphisms in susceptibility and clinical characteristics of prostate cancer*

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Abstract

Objective Recent studies have shown abnormal expression of octamer-binding transcription factor 4 (OCT4) and interleukin-18 (IL-18) to be related to cancer. However, the molecular mechanisms by which the IL-18 and OCT4 gene polymorphisms are associated with prostate cancer remain unclear. In this study, we aimed to determine whether the presence of IL-18 and OCT4 polymorphisms were associated with size, grade, tumor, nodes and metastasis (TNM) stage, or survival in patients with prostate cancer.

Methods Polymorphisms in OCT4 and IL-18 genes were evaluated to determine susceptibility to prostate cancer in 120 patients. A control group consisted of 125 Chinese participants. Genotyping was performed using TaqMan allelic discrimination assays, and statistical analysis was performed using SPSS.

Results No association was found between OCT4 and IL-18 gene polymorphisms and prostate cancer susceptibility. For OCT4 AA and IL-18-607 CC genotypes, there was a significant association with higher tumor grade (P = 0.03 and P = 0.025) and stage (P = 0.04 and P = 0.001). The OCT4 and IL-18-137 GG genotype was correlated with higher tumor grade (P = 0.028) and stage (P = 0.008). Furthermore, OCT4 AA was significantly more frequent in patients with lymph node metastasis (P = 0.02) and distant metastasis (P = 0.01). The Cox proportional hazard model showed that tumor grade and stage grouping were independent prognostic factors but IL-18 and OCT4 polymorphisms were not.

Conclusion The OCT4 gene may have a profound effect on prostate cancer risk. Polymorphism variants in the IL-18 (IL-18-607 and IL-18-137) and OCT4 genes may be associated with poor prognoses for individuals with prostate cancer.

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Key words: clinical characteristics; interleukin-18 (IL-18); octamer-binding transcription factor 4 (OCT4); polymorphism; prostate cancer

Initially, most patients with prostate cancer respond favorably to anti-androgen treatments or surgery. However, tumors frequently recur and progress towards the castration-resistant (CR) stage; for which therapeutic options are scarce. It has been suggested tumor initiation and progression is driven by small populations of cells endowed with stem-like properties: cancer stem cells (CSCs) [1]. Interestingly, CSCs may share properties, such as utilization of molecular pathways typically used by pluripotent embryonic stem cells (ESCs), with normal stem cells [1–2]. The prognostic significance of ESC gene expression signatures in solid tumors, including prostate cancer, has been successfully demonstrated [2–4]. Stem cell-like pluripotency has been successfully induced in differentiated fibroblasts upon reprogramming by transfecting a limited number of genes, including octamer-binding transcription factor 4 (OCT4) and Nanog [5]. Octamer-binding transcription factor 4 is a key transcription factor required to maintain the self-renewal and pluripotency of embryonic stem cells, and it enhances

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tumorigenesis in CSCs [6]. Increased expression of OCT4 is associated with low differentiation, tumor, nodes and metastasis (TNM) staging, and tumor recurrence in certain types of cancer, making OCT4 a promising biomarker for the diagnosis and prognosis of cancer in patients [7–9].

Recently, research showed that prostate tumor cells could secrete interleukin-18 (IL-18) in response to IFN-γ in the tumor microenvironment and that IL-18 could function as an autocrine or paracrine factor for the tumor [10]. Previously, we suggested IL-18 may play an important role in prostate cancer growth and metastasis, and we found that it correlated with serum IL-18 and VEGF in patients with prostate cancer. IL-18 proangiogenic functions are essential for tumor growth [11–12]. The IL-18 gene is located on chromosome 11q22 and functional gene polymorphisms -607A/C and -137G/C are found in its promoter region [13]. Our researchers found a change from C allele to A allele at position -607 and a change from G to C at position -137 of the IL-18 promoter region in prostate cancer patients [14]. These findings suggest IL-18 acts as a direct regulator of the self-renewal capacity of CSCs; however, the exact role of IL-18 in the regulation of CSC characteristics is not fully understood.

To further understand the role of IL-18 we recruited 245 participants, consisting of 120 patients with prostate cancer and 125 healthy individuals. The goal was to determine whether IL-18 and OCT4 gene polymorphisms, and their interaction with prostate cancer-related risk factors, are associated with susceptibility and clinicopathological development of prostate cancer among Chinese men.

Patients and methods

Patients

A total of 120 patients with prostate cancer who had undergone a radical prostatectomy between 2005 and 2011 at the Department of Urological Surgery, The Affiliated Hospital of Nantong University in China were evaluated. We excluded patients with infectious diseases and diabetes mellitus in order to eliminate the influence of other diseases. None of the patients with prostate cancer had received chemotherapy, hormonal therapy, or radiotherapy before surgery. Patient age ranged from 58–85 years and included 80 non-metastatic and 40 metastatic cases. The tumor stage was classified according to Whitmore-Jewett stage and was graded according to Gleason score. Patients were divided into low (≤ 6) and high (> 6) Gleason scores. Patient and tumor characteristics are listed in Table 1. Bone metastases were assessed by bone x-ray and bone scan, and extraosseous metastases were assessed by surgical biopsy. Recurrence was defined as a significant elevation of prostate-specific antigen (PSA) and/or new symptoms due to local tumor recurrence. The control group was comprised of 125 healthy volunteers who visited the general health check-up division at The Affiliated Hospital of Nantong University. Selection criteria for controls were no evidence of any personal or family history of cancer or other serious illnesses. Follow-up time ranged from 6 to 38 months with a median of 16 months after surgery. This study was performed with the approval of the ethics committee of Chinese Human Genome.

Table 1 Clinicopathological characteristics of prostate cancer patients

Characteristics	*n*	%
Age (years)		
Mean	70.43 ± 11.14	
Range	58–85	
Tumor stage		
A	5	4.2
B	67	55.8
C	10	8.3
D	38	31.7
Lymph node metastasis		
Negative	70	58.3
Positive	50	41.7
Metastasis		
Negative	80	66.7
Positive	40	33.3
Grade		
≤6	63	52.5
>6	57	47.5

DNA extraction

Genomic DNA was extracted from EDTA-anticoagulated peripheral blood leukocytes by the salting-out method. Blood (5 mL) was mixed with Triton lysis buffer (0.32 M sucrose, 1% Triton X-100, 5 mM $MgCl_2$, H_2O, and 10 mM Tris-HCl, pH 7.5). Leucocytes were spun down and washed with H_2O. The pellet was incubated with proteinase K at 56 °C and subsequently salted out at 48 °C using a saturated NaCl solution. Precipitated proteins were removed by centrifugation. The DNA in the supernatant fluid was dissolved in 300 mL H_2O.

IL-18 genotype

The genotyping of the two IL-18 polymorphisms was performed using predesigned TaqMan SNP Genotyping Assays (Applied Biosystems, USA). The Assays-on-Demand SNP genotyping kit (Applied Biosystems, USA) was used for the PCR. Single-nucleotide polymorphism (SNP) amplification assays were performed according to the manufacturer's instructions. A 25 μL sample of reaction solution containing 10 ng of DNA was mixed with 12.5 μL of 2x TaqMan Universal PCR Mix (Applied Biosystems, USA) and 1.25 μL of predeveloped assay reagent from the SNP genotyping product (Applied

Biosystems, USA), containing two primers and two TaqMan MGB probes. Reaction conditions consisted of preincubation at 50 °C for 2 min and at 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s and at 60 °C for 1 min. Amplifications and analysis were performed in an ABI Prism 7500 Sequence Detection System (Applied Biosystems, USA), running SDS 1.4 software for allelic discrimination (Applied Biosystems, USA). The following SNPs were typed: IL-18-137 G/C (rs187238) and IL-18-607 A/C (rs1946518).

OCT4 genotype

The TagSNPs were selected from the Haploview software 4.2 (Mark Daly's laboratory of Broad Institute, Britain) based on the GIH population data of HapMap (HapMap Data Rel 27 Phase II + III, Feb 09, on NCBI B36 assembly, dbSNP b126). TagSNPs that captured all known common SNPs (with minor allele frequencies of > 0.1) in the OCT4 genes, with a pairwise correlation r^2 > 0.8, were selected.

Statistical analysis

SNP allele frequencies were tested against departure from Hardy-Weinberg equilibrium before analysis. Genotype frequencies were compared using the Pearson χ^2 test for the 2 × 2 tables or Fisher's exact test when the expected frequency was $P < 0.05$. Patients were classified in a dichotomous manner for each of the following clinical parameters: tumor diameter, nuclear grade, tumor stage, lymph node metastasis, distant metastasis, stage grouping, and survival. The distribution of the polymorphism for each parameter was studied by analyzing genotype group and allele frequency. Odds ratios (ORs) and significance (*P*-values) were also calculated. The influence of each variable on survival was assessed by means of the Cox proportional hazard model. Values of $P < 0.05$ were considered significant. The SPSS statistical software package version 11.5 was used for all statistical analyses.

Results

Study subjects

The present study included 120 prostate cancer patients (mean age 70.43 ± 11.14 years) and 125 healthy controls (mean age 70.70 ± 9.41 years). The detailed baseline characteristics of the study subjects were given in Table 1.

Correlation of IL-18 gene polymorphisms with the clinicopathological characteristics prostate cancer

This case-control study revealed similar frequencies in the distribution of IL-18-137 and -607 polymorphisms between healthy controls and patients with prostate cancer. Table 2 presented the genotype distributions and statistical analysis. The observed genotype frequencies were in accordance with Hardy-Weinberg equilibrium. The association of the IL-18 genotypes with tumor grade and stage are shown in Table 3. Genotype GG of IL-18-137 was associated with more advanced cancer stage (OR: 2.61; 95% CI: 1.15–5.37; $P = 0.008$) and with higher tumor grade (OR: 3.32; 95% CI: 1.16–8.17; $P = 0.028$). IL-18-137 G allele was correlated with more advanced stage (OR: 1.73; 95% CI: 1.04–3.42; $P = 0.027$) and with higher tumor grade (OR: 2.13; 95% CI: 0.98–4.12; $P = 0.040$). The IL-18-607 CC genotype was significantly more frequent in patients with more advanced cancer stage (OR: 3.82; 95% CI: 1.67–7.67; $P = 0.001$) and higher tumor grade (OR: 3.11; 95% CI: 1.05–10.25; $P = 0.025$). The IL-18-607 C allele was associated with more advanced cancer stage (OR: 2.37; 95% CI: 1.28–3.73; $P = 0.001$). The association of the IL-18 genotypes with lymph node metastasis and distant metastasis are shown in Table 4. The IL-18-137 G allele was significantly more frequent in patients with lymph node metastasis (OR: 3.82; 95% CI: 0.95–15.17; $P = 0.035$).The IL-18-607 CC genotype was associated with distant metastasis (OR: 2.71; 95% CI: 1.25–6.14; $P = 0.025$).

Correlation of OCT4 gene polymorphisms with clinicopathological characteristics of prostate cancer

The observed genotype frequencies of the OCT4 gene polymorphisms studied in healthy controls were in accordance with Hardy–Weinberg equilibrium. No significant differences were observed in the frequency distribution of OCT4 polymorphisms between prostate cancer patients and healthy controls, both at the genotypic and allelic levels (Table 5). Genotype AA of

Table 2 Association of IL-18 genotypes with tumor risk (*n*, %)

IL-18 Polymorphisms	PC patients	Healthy controls	Odds ratio (95% CI)	*P*
-137 C/G Genotype				
CC	6 (5.0)	10 (8.0)	1.55 (0.45–4.05)	0.522
CG	47 (39.2)	40 (32.0)	1.00 (Reference)	
GG	67 (55.8)	75 (60.0)	0.78 (0.47–1.15)	0.725
Allele				
C	56 (23.3)	75 (30.0)	1.00 (Reference)	
G	184 (76.7)	175 (70.0)	1.45 (0.75–1.87)	0.072
-607 A/C Genotype				
AA	13 (10.8)	10 (8.0)	1.22 (0.56–1.96)	0.657
AC	61 (50.8)	65 (52.0)	1.00 (Reference)	
CC	46 (38.3)	50 (40.0)	0.787 (0.61–1.33)	0.322
Allele				
A	114 (47.5)	110 (44.0)	1.00 (Reference)	
C	126 (52.5)	115 (46.0)	1.36 (0.81–1.69)	0.381

CI, confidence interval; χ^2 Test or Fisher's exact test

Table 3 Association of IL-18 genotypes with tumor stage and grade

IL-18 Polymorphisms	Tumor stage (*n*, %) A–B	Tumor stage (*n*, %) C–D	Odds ratio (95% CI)	*P*	Tumor grade Odds (*n*, %) ≤ 6	Tumor grade Odds (*n*, %) > 6	Ratio (95% CI)	*P*
-137 C/G Genotype								
CC	4 (5.6)	2 (4.2)	1.25 (0.21–7.81)	0.625	3 (4.7)	3 (5.3)	1.36 (0.15–16.27)	0.620
CG	35 (48.6)	14 (29.2)	1.00 (Reference)		35 (55.6)	11 (193)	1.00 (Reference)	
GG	33 (45.8)	32 (66.7)	2.61 (1.15–5.37)	0.008	25 (39.7)	43 (75.4)	3.32 (1.16–8.17)	0.028
Allele								
C	35 (29.2)	32 (24.8)	1.00 (Reference)		55 (42.3)	32 (26.2)	1.00 (Reference)	
G	85 (70.8)	97 (75.2)	1.73 (1.04–3.42)	0.027	75 (57.7)	90 (73.7)	2.13 (0.98–4.12)	0.040
-607 A/C Genotype								
AA	8 (11.1)	3 (6.2)	0.83 (0.26–2.37)	0.617	5 (7.9)	4 (7.0)	1.17 (0.27–5.05)	0.782
AC	44 (61.1)	21 (43.8)	1.00 (Reference)		44 (69.8)	29 (50.9)	1.00 (Reference)	
CC	20 (27.8)	24 (50.0)	3.82 (1.67–7.67)	0.001	4 (22.2)	24 (42.1)	3.11 (1.05–10.25)	0.025
Allele								
A	57 (43.2)	45 (35.4)	1.00 (Reference)		35 (26.9)	23 (17.7)	1.00 (Reference)	
C	75 (56.8)	82 (64.6)	2.37 (1.28–3.73)	0.001	95 (73.1)	107 (82.3)	1.78 (0.87–4.52)	0.153

Table 4 Association of IL-18 genotypes with lymph node metastasis, metastasis, and stage grouping

IL-18 Polymorphisms	Lymph nodemetastasis (*n*, %) Negative	Lymph nodemetastasis (*n*, %) Positive	Odds ratio (95% CI)	*P*	Metastasis (*n*, %) Negative	Metastasis (*n*, %) Positive	Ratio (95% CI)	*P*
-137 C/G Genotype								
CC	2 (2.9)	1 (2.0)	0.84 (0.88–1.12)	0.672	2 (2.5)	1 (2.5)	2.81 (0.32–13.27)	0.427
CG	30 (42.9)	23 (46.0)	1.00 (Reference)		35 (43.8)	7 (17.5)	1.00 (Reference)	
GG	38 (54.2)	26 (52.0)	1.79 (0.33–8.35)	0.436	43 (53.7)	32 (80.01)	1.98 (0.92–5.17)	0.163
Allele								
C	65 (43.3)	5 (16.7)	1.00 (Reference)		42 (25.1)	11 (16.2)	1.00 (Reference)	
G	85 (56.7)	25 (83.3)	3.82 (0.95–15.17)	0.035	125 (74.9)	57 (83.8)	1.57 (0.82-3.50)	0.317
-607 A/C Genotype								
AA	3 (4.3)	2 (4.0)	0.87 (0.89-1.03)	0.343	5 (6.3)	2 (5.0)	2.47 (0.67-7.15)	0.168
AC	40 (57.1)	16 (32.0)	1.00 (Reference)		47 (58.7)	15 (37.5)	1.00 (Reference)	
CC	27 (38.6)	32 (64.0)	2.62 (0.68–9.67)	1.52	28 (35.0)	23 (57.5)	2.71 (1.25-6.14)	0.025
Allele								
A	60 (46.2)	5 (18.5)	1.00 (Reference)		61 (34.7)	21 (28.8)	1.00 (Reference)	
C	70 (53.8)	22 (81.5)	2.98 (0.89–8.93)	0.057	115 (65.3)	52 (71.2)	1.45 (0.83-3.45)	0.237

Table 5 Association of Oct4 genotypes with tumor risk (*n*, %)

IL-18 Polymorphisms	PC patients	Healthy controls	Odds ratio (95% CI)	*P*
Genotype				
AA	15 (12.5)	10 (8.0)	1.28 (0.54–1.95)	0.648
AC	61 (50.8)	64 (51.2)	1.00 (Reference)	
CC	44 (36.7)	51 (40.8)	0.877 (0.751–1.62)	0.412
Allele				
A	110 (45.8)	105 (46.7)	1.00 (Reference)	
C	130 (54.2)	120 (53.5)	1.26 (0.97–1.89)	0.401

CI, confidence interval; χ^2 Test or Fisher's exact test

OCT4 was associated with more advanced cancer stage (OR: 1.40; 95% CI: 0.62–3.42; $P = 0.04$) and with higher tumor grade (OR: 0.81; 95% CI: 0.41–1.82; $P = 0.03$).The OCT4 Genotype AA was significantly more frequent in patients with lymph node metastasis (OR: 4.08; 95% CI:1.42–10.12; $P = 0.02$) and distant metastasis (OR: 1.81; 95% CI: 0.81–3.42; $P = 0.01$), shown in Table 6.

Polymorphisms (IL-18 and OCT4) in cancer survival

Thirty-four patients died of cancer-related causes during the follow-up period. Fig. 1 and Fig. 2 showed the Kaplan-Meier curves calculated for cancer-specific survival for the IL-18-607 genotype (AC and CC) and OCT4 genotype (TT and CT + CC). Patients with the AC genotype showed a tendency towards more favorable cancer-specific survival than those with the CC genotype ($P = 0.075$; log-rank test). A Cox proportional hazard model demonstrated that tumor grade and stage grouping

Table 6 Correlation of the Oct4 gene polymorphisms with prostate cancer clinicopathological characteristics (*n*, %)

Oct4	AA	AC + CC	OR (95% CI)	*P*	A	C	OR (95% CI)	*P*
Clincal stage								
A + B	41 (59.4)	31 (60.8)	1 (Reference)	0.04	68 (56.7)	81 (67.5)	1 (Reference)	0.55
C + D	28 (41.6)	20 (39.2)	1.40 (0.62–3.42)		52 (43.3)	39 (32.5)	1.18 (0.62–2.25)	
Grade								
≤ 6	32 (54.2)	31 (51.8)	1 (Reference)	0.03	61 (50.8)	67 (55.8)	1 (Reference)	0.35
> 6	27 (45.8)	30 (49.2)	0.81 (0.41–1.82)		59 (49.2)	53 (44.2)	0.45 (0.39–1.21)	
lymphy node								
Negative	30 (57.7)	40 (58.8)	1 (Reference)	0.02	65 (54.2)	73 (60.8)	1 (Reference)	0.39
Positive	22 (42.3)	28 (41.2)	4.08 (1.42–10.12)		55 (45.8)	47 (39.2)	1.16 (0.45–2.17)	
Metastasis								
Negative	27 (56.3)	57 (75.0)	1 (Reference)	0.01	83 (69.2)	76 (63.3)	1 (Reference)	0.28
Positive	21 (43.7)	19 (25.0)	1.81 (0.81–3.42)		37 (30.8)	44 (36.7)	0.66 (0.25–1.71)	

Table 7 Multivariate analysis of overall survival in prostate cancer patients

Variable	B	SE	Wald	*df*	*P*	Exp (B)
Tumor grade	1.433	0.701	5.253	1	0.035	3.476
Tumor Stage	1.575	0.527	15.217	1	0.002	4.612
IL18 -137	–1.673	1.132	4.076	1	0.073	0.180
IL18 -607	0.415	0.507	0.517	1	0.511	1.415

were independent prognosis factors (Table 7). However, IL-18 polymorphisms, at least in this series of patients, did not serve as independent prognosis factors.

Discussion

CSCs are important in carcinogenesis and resistance to treatment and may lead to metastasis. The isolation of circulating stem cells involves cell sorting based on the presence of cell surface markers. The cell surface marker, OCT4 has been reported to be overexpressed in colorectal cancer and expression has been observed in bladder cell cancers [15–16]. Considering the role of OCT4 as a pluripotency factor, and possible role in the etiology of cancer, OCT4 was investigated as a marker for CSCs. Expression of OCT4 has been reported previously in benign prostate and cancer cell lines as evidence of CSC compartments. A subpopulation of telomerase-immortalized prostate epithelial cells that demonstrated stem cell properties expressed OCT4 protein [17]. Similarly, subpopulations of prostate cancer cells that were capable of reconstituting the original prostate tumor in vivo expressed OCT4 mRNA in cultures [18]. These observations suggest OCT4 is a marker for prostate CSCs. Therefore, in order to elucidate the role of OCT4 polymorphisms in cancer; polymorphisms in human prostate cancer were assessed.

Cytokine IL-18 is known to play a critical role in the development and progression of tumors, including prostate cancer tumors. Our results showed a strong association between increased expression of IL-18 and poor outcome in prostate cancer patients. Experimental studies have demonstrated IL-18 may promote

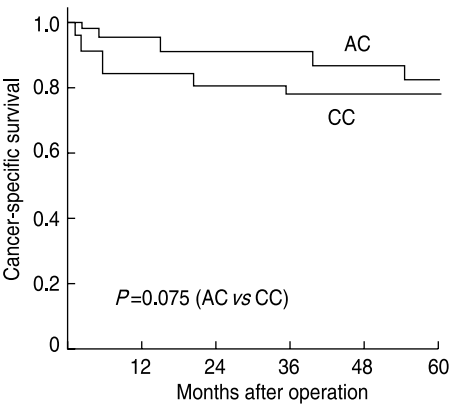

Fig. 1 Kaplan-Meier overall survival estimate for IL-18-607 polymorphism. Differences between curves were evaluated by log-rank test [*P* = 0.075 (AC *vs* CC)]

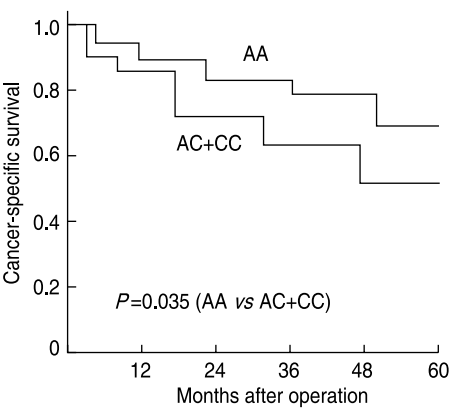

Fig. 2 Kaplan-Meier overall survival estimate for OCT4 polymorphism. Differences between curves were evaluated by log-rank test [*P* = 0.035 (AA *vs* AC + CC)]

tumorigenesis, angiogenesis, and metastasis, and induce multi-drug resistance in cancer cell lines [19–21]. Moreover, emerging evidence suggests IL-18 has an important role in CSC phenotype and function. Finally, IL-18 has been found to enhance the tumorigenicity in glioblastomas, which is consistent with the increased capacity of CSCs to self-renew [22–23]. These findings suggest that IL-18 may act as a direct regulator of the self-renewal capacity of CSCs; however, the exact role of IL-18 in the regulation of CSC characteristics is not fully understood.

In the present study, we found no association between IL-18 and OCT4 polymorphisms and a higher risk of prostate cancer. However, as shown in other studies these polymorphisms were correlated with more advanced cancer stages [24–25]. Some studies have suggested IL-18 promoter polymorphisms are associated with prostate cancer and prostate cancer risk, although this was contradicted by other studies [26–28]. Our findings support the recent suggestion that pleiotropic cytokine IL-18 can exert both an anticancer and procancer influence [29]. In fact, IL-18 activities are influenced by the tumor microenvironment. So, IL-18 may exert antitumor activity by augmenting IFN-γ production, particularly in the presence of IL-12 [29]. However, recent data suggest a procancer activity for this multifunctional cytokine under certain conditions depending on the tumor immune response at different tumor sites, and genetic background [30]. Polymorphisms (IL-18 and OCT4) do not appear to be associated with prostate cancer susceptibility in our participants. This may be attributable to the different genetic backgrounds and environmental factors, such as different carcinogens, that initiate different cancers, and different carcinogen exposure. In addition, inadequacies in study design, such as nonrandom sampling and a limited sample size, should be considered. Selection bias in this hospital based, case-control study must also be considered. Finally, we cannot ignore that the observed association is dependent on linkage disequilibrium in the IL-18 gene, or on the effect of IL-18 on another peptide.

We found that a genotype related to higher production of IL-18 is associated with higher grade and stage of the tumor. IL-18 activates HIF (hypoxia-inducible factor-1alpha) and vascular endothelial growth factor, and may activate angiogenesis in tumor nests [29, 31]. Therefore, IL-18 polymorphisms may increase angiogenesis and provide adequate nutrients to transformed cells, promoting more advanced stage. Progression is also correlated with IL-18. High-production polymorphisms in IL-18 are associated with dedifferentiation of tumor cells, leading to a more advanced tumor grade and stage grouping. Elevated IL-18 expression was found to be correlated with the malignancy of skin cancers and with the progression of breast cancer [24, 32]. Therefore, IL-18 can directly promote proliferation by regulating proliferation stimulators. IL-18 was recently implicated in the migration of lung cancer and human melanoma cell lines through the region of interest generation method in mitogen-activated protein kinase pathway [33–34]. Our results were similar to previous findings that proinflammatory cytokines induce adhesion receptors of endothelial cells for cancer cell attachment, which is necessary for blood-borne metastasis [35]. The clinical importance of these parameters is worth investigating in patients with prostate cancer, especially for patients with bone metastasis; however, larger studies are needed. In the present study, polymorphisms related to IL-18 production were associated with the development of metastasis and lymph node involvement.

Our study revealed that the expression of OCT4 was correlated with tumor size and lymph node metastases. This study, and other research, may indicate an association between OCT4 nuclear accumulation and tumorigenesis [36]. In addition, OCT4 is more frequently located at the invasive front of tumors and correlates significantly with various aggressive behaviors and epithelial-mesenchymal transition (EMT) in nasopharyngeal carcinoma [37]. The expression of OCT4 in melanoma cells increases transmigration capacity, leading to high invasiveness and aggressiveness, while promoting cancer cell proliferation and formation [38–39]. Inversely, knockdown of OCT4 inhibits CSC cell motility and invasion and decreases hepatic colonization [40]. Patients with low OCT4 expression exhibit an improved overall survival rate [41]. This study provides support that polymorphisms related to OCT4 production were associated with the development of metastasis and lymph node involvement, suggesting OCT4 may be an effective therapeutic target for the treatment of cancer.

The association between overall survival and IL-18-607 polymorphism was also analyzed. Because the median survival (50% mortality) was not achieved, we cannot comment on the statistical influence of this variable as a prognostic factor. Although, polymorphisms related to IL-18 production were strongly correlated with more advanced stages of prostate cancer, explaining increased mortality (P = 0.076). Cox analysis revealed IL-18 and Nanog polymorphisms are not independent risk factors for mortality. We propose that the influence of IL-18-607 polymorphism is more significant than that of IL-18-137, promoting high-risk phenotypes.

Cancer stem cells have been found to be regulated by mesenchymal stem cells through cytokine networks, including IL-6 and IL-8 [42]. Recent studies have demonstrated colon CSCs promote tumor formation and growth through the autocrine effect of certain cytokines, such as IL-8 and IL-4 [43–44]. A paracrine effect of mesenchymal stem cells in promoting tumor growth of CSCs by secreting cytokine IL-6 has also been revealed [45]. Additionally, IL-6 has been shown to enhance

tumorigenicity in glioblastoma, consistent with an increase in the CSCs self-renewal capacity [46]. Here, we show for the first time, the role of the combination of IL-18 and OCT4 gene polymorphisms in susceptibility, and clinical characteristics of prostate cancer. We found that polymorphisms of IL-18 and OCTt4 are associated with higher grade and stage of the tumor, development of metastasis, and lymph node involvement. These findings provide evidence to support that IL-18 may function as a direct mediator of CSCs self-renewal capacity. However, the exact role of IL-18 in the regulation of CSC characteristics requires further investigation.

Conflicts of interest

The authors declare no conflicts of interest.

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